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**Behavioural consequences of visual deprivation occurring early or later in the life of
chickens**

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25 ABSTRACT

26 The development of behaviour in a line of chickens that are born sighted (*rdd*) but
27 turn blind after hatching was compared with a line that is blind at hatch (*beg*) and with
28 sighted White Leghorn controls (WL) to test the hypothesis that birds that become blind later
29 in their life will show intermediate behavioural disturbances compared to animals that are
30 blind from hatch. Individual behaviour, group aggregation and behavioural synchrony were
31 compared at 1, 5 and 9 weeks of age (experiment 1) and in the parents of these chicks at 9-13
32 months of age (experiment 2). Responses to visual and physical isolation were assessed at 1,
33 5 and 9 weeks of age.

34 Analyses of home-pen behaviour showed that *rdd* and *beg* had difficulty locating or
35 consuming food. WL and *rdd* did not engage in abnormal behaviour at 1, 5 and 9 weeks of
36 age whereas both *beg* and *rdd* adults did so. At 9 weeks of age *beg* and *rdd* birds showed
37 decreased behavioural synchrony compared with WL, whereas group aggregation in *rdd* and
38 WL was similar and higher than in *beg*. WL adults showed increased environmental pecking
39 and higher rates of behavioural synchrony and group aggregation than both *beg* and *rdd*.
40 Under visual isolation from conspecifics *rdd* behaved like blind birds in some respects (e.g.
41 decreased movement) and as sighted birds in others (e.g. peeping). Contrary to previous
42 reports, the vision of *rdd* was apparently diminished compared with sighted controls (WL) at
43 all ages.

44 It was concluded that abnormal behaviours are a response to a complete loss of vision
45 regardless of initial sight. Birds that became blind during rearing (*rdd*) may be more active as
46 adults than birds that were blind throughout life but in general the behaviour of blind birds
47 was similar regardless of early sight.

48

49 *Key words:* chicken; blindness; sight; social isolation; behaviour

1. Introduction

Vision is important in birds and their highly specialised visual systems mediate the majority of their behaviours including feeding and drinking, navigation and social behaviour including dominance and courtship (Collins et al., 2011). Chickens have particularly well developed visual abilities and vision is thought to play a crucial role in the acquisition, development and maintenance of a number of normal behaviours through the use of visual cues (Mench and Keeling, 2001; Prescott et al., 2003). Lack of vision is therefore likely to compromise the development of their behaviour due to the absence of environmental sensory stimulation, and their ability to express key behaviours. A line of chickens with the genotype *beg* (blindness, enlarged globe) are blind from hatch and show less group aggregation and behavioural synchrony than sighted controls (Collins et al., 2011). Furthermore the blind birds exhibited abnormal behaviours, grew less well than sighted counterparts and tended to have greater mortality (Collins et al., 2011).

Several issues regarding the importance of sight for the development of behaviour remain to be clarified: do the differences between the lines observed in young chicks, remain in adult birds? Can the results on abnormal and social behaviour be generalized to a genotype that develops blindness later in life? Can the problems of poor growth and higher mortality be ameliorated by the possession of sight at a young age and before becoming blind later in life?

The main aim of the current study was to investigate in detail the behaviour of chickens from the retinal dysplasia and degeneration (*rdd*) genotype that were sighted at hatch and become progressively blind until there is complete loss of vision at sexual maturity (Randall et al., 1983), and to determine how their behaviour changed once they lost their sight, compared with chickens from a genotype that were blind from hatch (*beg*) and normal sighted chickens. Our hypothesis is that birds that become blind later in their life will show intermediate behavioural disturbances compared to animals that are blind from hatch.

Chicks from the three lines were reared in separate pens to record behaviour in the home pen. Behavioural synchrony and group aggregation and a test of the effects of visual and physical isolation were also investigated. Body weight gain, mortality and heterophil-lymphocyte (HL) ratios were determined as proxy measures of essential functions behaviours and the welfare of blind birds. Stressors elevate the number of heterophils and depress the number of lymphocytes and HL ratios are less variable and longer lasting than corticosterone responses (Gross and Siegel, 1983). In a second experiment, adult females and males were moved from individual breeding cages into floor pens containing birds of the same line and gender. Behavioural observations, behavioural synchrony and social aggregation were recorded to compare the behaviour of the three lines when *rdd* were unequivocally blind.

2. Methods

2.1 Experiment 1: chicks 0 - 9 weeks of age

2.1.1 Animals and housing

A total of 72 White Leghorn-type birds of unknown gender were used for the experiment consisting of 24 White Leghorn (WL), 24 *beg* and 24 *rdd*. The WL chickens have normal sight and are genetically related to both *beg* and *rdd* by back crossing WL males into of the original lines.

The chicks were wing-banded and randomly allocated to one of 12 pens over two rooms after hatching. Each pen was 1.52 m wide and 2.10 m long with solid pen walls to 0.6 m and wire to 2.0 m. The aisle between the two blocks of pens was 1.60 m and there were two blocks of three pens in both rooms. Each pen contained 6 birds of one of the three lines. The birds were identified by wing-bands and given a unique symbol on the head and back

with a black marker pen for ease of overhead identification during the observation period. Birds were penned with those of the same genotype but the gender was not known until the completion of the experiment when the gender of the birds was determined retrospectively by visual inspection of size and color of the comb and wattles.

There were a number of spare birds of each line that were kept in room 1 in an extra pen, under the same conditions as the study birds, in case replacement was required. In the first 7 days, 7 *beg* chicks and 1 *rdd* chick died, and, in week 2, one WL chick died. These birds were replaced with randomly selected chicks of the same line from the stock of extra chicks.

The birds had *ad libitum* access to food and water in the home pens. They were fed a standard layer chick crumb from hatch to 6 weeks of age followed by a pelleted grower feed. The food was provided in large, shallow chick pans on the floor of each pen throughout the experiment to minimise the risk that feeding in the *beg* chicks might be compromised by the change to a hanging tube feeder. Water was available from bell drinkers placed on the floor and later from larger suspended drinkers. The temperature of the rooms was 29°C at hatch and was gradually decreased to 22°C at 4 weeks of age. Heat lamps were used to provide extra warmth until the chicks were 4 weeks of age. The photoperiod was 14 hours per day from 07:00 - 21:00 h. The average light intensity at chick eye level in room 1 was 77 lux, and 50 lux in room 2.

Since it is known that newly hatched *beg* chicks often have trouble locating food (Pollock et al., 1982), for the first week, the *beg* chicks were assisted to find feed and water and some were fed and watered by hand. Curved hardboard surrounds were also placed at the back corners of the pens to encourage the chicks towards the food and water.

2.1.2. Home pen behaviour

128 Video cameras were positioned above each pen to record the birds' behaviour. There were 6
 129 VCRs and video recording was conducted over two days of one block in each room followed
 130 by the other block on the subsequent day at 1, 5 and 9 weeks of age. On each day, the pens
 131 were recorded for three 1-hour periods from 09:00-10:00 h, 13:00-14:00 h and 17:00-18:00 h.
 132 From the video tapes, 5-minute instantaneous scan sampling (Martin and Bateson, 1993, pp
 133 85-86) was used to record the frequency of behaviours (%) in the pens by one person, who
 134 was blind to the treatment, using the ethogram in Table 1. To quantify group aggregation,
 135 mean nearest neighbour distance (NND; (2009)) was recorded at successive 5-minute
 136 intervals using digital callipers to measure the distance (mm) between each bird and the
 137 nearest part of its closest pen mate on the video monitor screen. Behavioural synchrony (Bs)
 138 was calculated at each interval using Simpson's Diversity Index (King and Cowlishaw, 2009)
 139 as follows for each behavioural scan:

$$Bs = \frac{\sum n_i(n_i - 1)}{N(N - 1)}$$

140 where n_i is the number of individuals exhibiting the i^{th} behaviour and N is the total number of
 141 individuals visible in the scan.

142 Since some of the behaviours were observed to be performed by the chicks very
 143 infrequently, the behavioural categories were merged into 9 classes: feeding, drinking, sitting,
 144 standing, walking and running, environmental pecking, preening, abnormal (circle walking,
 145 air pecking, star gazing), and other (lie, gentle feather peck, severe feather peck, aggressive
 146 peck, dust bathe, stretch, chase and display). Mean time spent carrying out each behaviour
 147 was calculated for each pen, time period and week, and expressed as a proportion of the birds
 148 in view.

149

150 2.1.3 Social Isolation

151

152 The social isolation tests were performed as described by Collins *et al.*, (2011). Briefly, a
153 testing arena was divided into a ‘test side’ and a ‘companion side’ by a divider that was either
154 a wire mesh (physical isolation) or a wooden panel (visual isolation). The subject was placed
155 in the test side, while its pen mates remained in the companion side. The test side contained a
156 wooden ‘start box’ that was removed by the observer pulling on a rope and pulley system.
157 The start box was placed in the centre of the test side with one wall of wire mesh facing the
158 companion side. The chicks could not see the experimenter at any time during the recording
159 period and behavioural recording was conducted through a camera placed directly above the
160 test area.

161 Testing occurred between 10:00 and 18:00 h. The pens were selected in a predefined
162 random order from each block and all six birds were placed in the companion side of the
163 arena for 1 min to habituate to the surroundings. The birds were then tested in a predefined
164 random order. Each bird was given 1 min to habituate to the start box and the test lasted for 2
165 min. When all 6 birds in the pen had been tested, they were returned to the home room and
166 the next group was collected. All chicks were tested under both physical and visual isolation
167 treatments over two days, and to avoid potential order effects, genotype and isolation
168 treatment were balanced over these two days. To estimate the importance of a carryover
169 effect, the trials from day 2 were repeated on day 3. The same predefined random order of
170 pens and birds was used at weeks 1, 5 and 9.

171 For each chick, the 2 min videos were used to record the latency to move (s), number
172 of pecks, proximity to pen mates (all putative measures of sight), number of peeps (a
173 potential measure of frustration) and number of lines crossed (a measure of activity). The
174 lines crossed refer to a 25 cm x 25 cm grid on the test side floor that was videoed and
175 transferred to an acetate sheet placed over the video monitor screen. Proximity to pen mates
176 was recorded by 10 s instantaneous sampling (Martin and Bateson, 1993, pp 87-88); test
177 chicks were recorded as ‘near’ to pen mates if located in one of the 4 grid squares closest to

178 the divider and as ‘far’ if in one of the other 4 squares. If the chick was positioned in more
179 than one square of the grid, the square that its beak was in was recorded. All observations
180 were conducted by one person who was blind to the treatment of the birds.

181

182 *2.1.4. Body weight, mortality and heterophil:lymphocyte (HL) ratio*

183

184 All birds were weighed to the nearest gram (g) on the day of hatch, and every week
185 thereafter. The date of death of any bird was recorded and overall mortality of the three
186 genotypes was assessed. A drop of blood was obtained at 5 and 9 weeks of age by inserting a
187 needle (orange 25G) into a wing vein. A blood smear was immediately prepared on a glass
188 slide, air dried and subsequently stained using May-Grünwald’s solution as previously
189 described (Robertson and Maxwell, 1990). The numbers of heterophils and lymphocytes in
190 100 cells were counted and the ratio (HL) calculated.

191

192 *2.1.5. Tests for blindness*

193

194 During week 3, a basic test was carried out to assess if the birds could see or not. A hand was
195 moved at a distance of 10-20 cm from each eye and recording whether or not it reacted by
196 moving its head or walking away. At the start of week 6, all birds were again tested on their
197 ability to see. Three different assessments were carried out; i) the box test, ii) the “hawk” test,
198 and iii) the laser test. In the box test, for each pen all 6 birds were placed in a cardboard
199 transport box. They were left in the box for 1 min to habituate. After 1 min, the box was
200 opened and a stopwatch was started. The following were recorded: the time (latency) to exit
201 the box for the first and last bird and the number of birds that had not left the box after 3 min.
202 In the “hawk” test, a black cardboard outline of a hawk attached to a wooden pole was
203 “swooped” over each pen. The number of birds that initially responded (either by freezing or

running away) was recorded. The third test was conducted with a red laser presentation pointer by the observer standing outside of the pen. The light was shone on the ground near each bird and moved around; the number of birds that responded by either pecking or running away in each pen was recorded. At the end of week 9, the laser test was repeated since it appeared to be the most accurate for assessing blindness.

2.2. Experiment 2: adult chickens

2.2.1. Animals and housing

The birds for this experiment were reared under similar conditions as experiment 1 and were housed in individual laying cages (800 x 450 mm) at 16 weeks of age for pedigree breeding. They were rehoused to floor pens (1.5 x 2.2 m) when they were between 9 and 13 months of age. The birds were allowed to familiarise themselves with the pen environment for 6 weeks before behaviour recording. A standard layer ration and water were available at all times and the photoperiod was 14L:10D (0730-2130h). Each pen was provided with a set of 3 nest boxes.

There were originally 8 males and 8 females in each line housed at random in each of 12 pens containing 4 males or 4 females of the same genotype. Line and sex were randomised to pen in a 3 x 2 completely randomised design with 2 replicates (blocks). The blindness of *rdd* was assessed by the red laser test as described in section 2.1.4.

2.2.2. Home pen behaviour

Video cameras were placed above the centre of each pen and the birds were video recorded for 1 h periods starting at 09:00h, 13:00h and 17:00h during 2 consecutive weeks. One

replicate from each breed-sex group was videotaped on day one and the second on day 2 in each week and again on the following week so that each pen was recorded for a total of 6 hours. The frequency of different behaviours, behavioural synchrony and group aggregation in the home pens was assessed from the video tapes as described in section 2.1.2.

2.3 Statistical analysis

The numbers of observations of each behaviour in every pen and hour were summed and analysed as a proportion of the total number of observations assuming binomial errors in the GLMM procedure of the Genstat statistical package (<http://www.vsni.co.uk/software/genstat>). For each category of behaviour, a nested analysis of a model including effects for block (4 rows) and pen within block as random factors and line, week, time of day and their interactions as fixed effects was conducted. Line x week x time interactions were not significant for any behaviour and the data were reanalysed with two-way interactions only. Line x time and time x week interactions were also not significant and a final model with line x week and time was analysed. The residuals for mean Bs and NND were normally distributed with approximately equal variance and the untransformed data for both measures were analysed using the same statistical model as behavioural frequency in the ANOVA procedure.

Mean pen latency to move (s), number of peeps, frequency of pecks, number of lines crossed and % of scans near pen mates were calculated for each pen in the isolation experiment for each combination of treatment, day and week. Latency to move was transformed by taking the logarithm ($x + 0.1$), the number of peeps and pecks by the square root ($x + 0.2$), the number of lines crossed by the square root (x) and the percentage (p) time near pen mates as the empirical logistic (logit). A nested analysis of variance was carried out

for each trait, with line, isolation, week day (1, 2, or 3), carryover from visual isolation on the previous day, and their interactions as the treatment effects.

The overall number of birds responding or not responding in the evaluation of sight in each line in experiment 1 was evaluated by an overall Chi-square test for each test and age.

Analysis of the weekly weights was performed by a REML analysis of a model with fixed effects for line, gender and their interaction. Mortalities only occurred in weeks 1 and 2. Pen mean mortality (number dead in first 2 weeks/total at start) was analysed by analysis of deviance assuming a binomial distribution of errors. A nested analysis of variance of the HL ratio was performed with treatment effects of line, time and their interaction.

For the analysis of behaviour in experiment 2, the GLMM procedure in Genstat was used to analyse a model with breed, gender, time and their 2- and 3-way interactions as fixed terms with block/pen/day as nested random effects. Data assumptions and transformations were as described for experiment 1 except that natural logarithms of NND were taken to ensure equality of the residual variance across treatments.

All differences between effects were supported by a level of statistical significance of at least $P \leq 0.05$.

2.4. Ethical note

The birds were kept at The Roslin Institute in Edinburgh under a Home Office licence (PPL/60/3815 protocol 2) after ethical review. The birds were from a breeding stock that is used for the study of potential animal models for human conditions and for basic research into the development of sight. The minimum numbers of breeding birds are kept to maintain the genetic lines without incurring unacceptable levels of inbreeding.

3. Results

3.1. Social behaviour

3.1.1. Frequency of behaviours in young chicks and adult chickens

Results for time of day were comparable with the well-known effects of time of day on the behaviour of chickens and are ignored for clarity of presentation. Relatively few interactions of main effects and time of day were significant and were also ignored as the effects were relatively small and conclusions for line and age were not affected. Back transformed means for each line by week are plotted in Fig. 1. Levels of statistical significance are indicated above the relevant column for tests of *beg* vs *rdd* and *rdd* vs WL. Means and SED on the transformed scale and statistical tests of significance for line, week and the line x week interaction are presented in Supplementary Table 1.

Observations of drinking were low in *beg* compared with *rdd* in week 1 and week 5 but were similar to WL at week 9. Feeding was relatively infrequent in week 1 in *beg* compared with *rdd* and was lower in *rdd* compared with WL but the means were similar at weeks 5 and 9. Abnormal behaviour was relatively high in *beg* at 5 and 9 weeks and rare in both *rdd* and WL. The ranking of the three lines for pecking at the environment was similar at 1, 5 and 9 weeks: differences between *beg* and *rdd* were significant at 5 and 9 weeks and *rdd* was different from WL at 9 weeks. Sitting behaviour was the most frequently observed behaviour in *beg* and *rdd* at ages. However at 9 weeks the frequency of sitting in *beg* was greater than in *rdd* and WL which were similar. Walking was relatively high in *rdd* at 9 weeks compared with both *beg* and WL.

Means and SED on the transformed scale and statistical tests of significance for line, gender and line x gender interaction for adult birds are presented in Supplementary Table 2. The effects of time of day were ignored as described above for the younger birds.

307 Backtransformed means for line and gender are plotted in Fig. 2 with tests for *beg* vs *rdd* and
308 *rdd* vs WL above the relevant columns.

309 The frequency of drinking was relatively high in female *rdd* adults but there were no
310 line differences in males. Feeding was higher in WL male adults compared with *beg* and *rdd*
311 which were similar and there were no differences in females. Abnormal behaviours were
312 observed only in *beg* and *rdd* in both sexes and differences between the blind birds were not
313 significant. Preening was observed more frequently in male *rdd* compared with *beg* and WL
314 which were similar. Standing and pecking the environment were the most frequently
315 observed behaviours. WL females pecked the environment more often than both blind lines
316 but differences in males were small and not significant. Standing was higher in *beg* females
317 compared with *rdd* and WL whereas standing in male WL was higher than in *rdd* and *beg*.
318 Conversely, walking was relatively low in male WL compared with *beg* and *rdd*. Nesting was
319 observed in WL but not in *rdd* or *beg* chickens which laid their eggs on the pen floor (data
320 not shown).

321

322 3.1.2. Behavioural synchrony and group aggregation

323

324 Means and test results for the analyses of variance for behavioural synchrony (Bs) and group
325 aggregation (NND) are presented in Tables 2 and 3 respectively for chicks (experiment 1)
326 and adults (experiment 2). Bs in chicks was greater in WL than in *beg* and *rdd* which were
327 similar and the behaviour of all three lines was more synchronous in week 1 than in weeks 5
328 and 9 (Table 2). In adult birds, sighted WL birds showed a significantly higher level of Bs
329 compared with both blind *rdd* and *beg* (Table 3).

330 There was a significant line x week interaction in the young chickens for NND since
331 in weeks 5 and 9, *rdd* and WL had similar NND that were significantly less than that of *beg*
332 (Table 2). In adult chickens, sighted WL males birds showed reduced NND compared with

males of the other two lines whereas no differences were found between the females leading to a line x gender interaction (Fig. 3). Main effect means and levels of significance for Bs and NND in the adult birds are presented in Table 3.

3.2. Social Isolation in young chicks

Statistically significant interactions of week, line, carryover effects and day were ignored for clarity of presentation as these were nuisance factors correctly accounted for in the analysis. Means and tests of significance on the transformed scale for the main effects and interactions are given in Supplementary Table 3.

Highly significant ($P < 0.001$) line x isolation interactions were detected for each trait and back transformed means for line x isolation subclasses are plotted in Fig. 4. The latency to move of *beg* chicks was greater in both isolation treatments compared with WL or *rdd* chicks (Fig. 4A). The latency to move of WL and *rdd* chicks was not significantly different from each other except under physical isolation where the latency to move by *rdd* was higher than in WL. Similarly, for both *beg* and *rdd*, the number of lines crossed did not differ between the two isolation treatments whereas for WL it was higher under physical compared with visual isolation (Fig. 4B). Under physical isolation, the WL chicks crossed more lines (i.e. were more active) than *beg* or *rdd* chickens, but under visual isolation, all three lines were similarly inactive.

The number of pecks was greater under physical than visual isolation for all three lines (Fig. 4C) and the number of pecks was greater in WL compared with *rdd*, and in *rdd* compared with *beg*. The number of peeps made by WL and *rdd* was greater in visual than physical isolation whereas for *beg*, the number of peeps was similar in both treatments. All three lines made a similar number of peeps under physical isolation (Fig. 4D).

For both WL and *rdd*, time spent near pen mates was significantly greater under physical isolation than visual isolation (Fig. 4E). Under physical isolation, both WL and *rdd* chicks spent a similar amount of time near pen mates, although the percentage for WL was statistically higher ($P<0.05$), and was greater than that for *beg*. Under visual isolation, time spent close to pen mates was also higher for WL compared with both *rdd* and *beg*.

A significant interaction ($P<0.05$) of genotype and age was detected for the number of pecks and proportion of scans near pen mates (Fig. 5). The number of pecks was similar in *rdd* and *beg* at all 3 ages whereas the number of pecks in WL was lower at 1 week and higher at 5 and 9 weeks compared with both *beg* and *rdd* (Fig. 5A). The percentage of time that *beg* chicks spent near pen mates was lower in *beg* than in *rdd* and in *rdd* compared with WL at 1 week only (Fig. 5B).

3.3. Tests of sight

Results for the number of birds responding to different tests of sight at 3, 6 and 9 weeks of age are presented in Table 4. As expected, *beg* showed no response to any test and WL responded maximally to all of them. The responses of *rdd* declined with age and a degree of sight remained at 9 weeks of age in at least 25% of these birds. However, the adult *rdd* birds that were parents of these chicks did not respond to the red laser light and were functionally blind.

3.4. Body weights, HL ratios and mortality in chicks

The average body weights of *beg*, *rdd* and WL chicks, sex-average body weight gain from 0-7 and 8-14 days and body weight at 10 weeks of age are given in Table 5. Differences between genders were significant from week 2 onwards and sex x line interactions were

significant from week 5 ($P < 0.05$): These were not significant when the data were logged, indicating that the interaction was a scale effect. Line means for the HL ratio and mortality are also presented in Table 5. Week and week x line had no detectable effect on the HL ratio whereas *rdd* had a very low ratio compared with both WL and *beg* ($P < 0.01$). Mortality in *beg* chicks was higher than in *rdd* and WL chicks during the first 2 weeks (Table 5) but there were no subsequent deaths during the course of experiment 1. In experiment 2, two adult *rdd* females, one male and one female *beg* died before the experiment started. Cannibalism of a WL female resulted in this bird being culled before the observations began and an additional WL female from the same pen was culled for cannibalism of the vent during the study.

4. Discussion

The main aim of this study was to study the behaviour of *rdd* birds and how this changed as their loss of sight progressed in comparison to the blind *beg* and sighted WL. It was hypothesised that for the first 3 weeks of life, while sighted, *rdd* would behave similarly to WL and continue to do so, at least to some degree, once they began to lose sight. The lack of abnormal behaviours in *rdd* in experiment 1 (Fig. 1) suggests that they were experiencing greater visual stimulation than *beg* even if their sight was compromised. However, as adults there were generally few differences between *rdd* and *beg* suggesting that the differences in the behaviour of blind birds is a consequence of their lack of sight and that there are in adulthood no significant long term effects of vision at an early age, or of behaviour that was learnt when the birds were sighted. The results emphasise the critical importance of vision for the display of normal behaviour. Whereas the *beg* and WL birds in the experiment of Collins et al. (2011) were genetically similar except for the genetic mutation (they were from a backcross), both *beg* and *rdd* chickens in this experiment were from parents which differed both from each other and from the parents of the WL. The similarities of the results for *beg*

410 and controls from both experiments suggest that comparisons in the present experiments are
411 valid tests of the effect of developing blindness in *rdd*. Furthermore the comparisons between
412 lines (state of vision) are made at the same age in identically treated birds.

413 The results from experiment 1 confirm previous observations that *beg* birds exposed
414 to social isolation showed reduced social reinstatement behaviour (Collins et al., 2011).
415 However, the behaviour of *rdd* was intermediate or more like one of the other two lines
416 suggesting that they were visually aware of the location of their conspecifics while being as
417 inactive as *beg* (Fig. 4 A, B, C and E). Vocalisation is strongly dependent on social contacts
418 in the chick (Marx et al., 2001) and the greater mean number of peeps for *rdd* compared with
419 WL and *beg* suggests that they were under greater stress from limited ability to see their
420 conspecifics compared with the blind or sighted line.

421 Social aggregation was greatest in week 1 for all three lines and reflects the fact that
422 the birds huddled together under the heat lamp. In contrast to behavioural synchrony, in
423 weeks 5 and 9, *rdd* and WL had significantly higher group aggregation (i.e. lower NND) than
424 *beg*. Behavioural synchrony and group aggregation are visually dependent and *beg* performed
425 poorly in this respect which is in line with previous results (Collins et al., 2011). Chickens
426 naturally carry out behaviours in synchrony and there are possibly strong social facilitation
427 and physiological effects motivating the birds to act in this way (Mench et al., 1986) and,
428 conversely, separation and isolation from social companions has been shown to be stressful to
429 chickens (Jones and Williams, 1992; Marx et al., 2001).

430 Both *beg* and *rdd* spent proportionally less time feeding at week one than the sighted
431 controls and differences in body weight and mortality in *beg* confirm previous suggestions
432 that chicks that are blind at hatch have difficulty learning to feed, have lower subsequent
433 growth rates and higher mortality during the first 2 weeks of life (Collins et al., 2011).
434 However the low rate of feeding and body weight of *rdd* was unexpected and may indicate
435 that their vision was compromised even though they were able to see sufficiently well to

436 locate feed and water and conduct normal behavioural activity, at least to 3 weeks of age.
437 Furthermore, the relative lack of interaction of line and age in the isolation experiment
438 suggests that the vision of *rdd* was compromised from an early age. In support of this
439 suggestion, degenerative changes associated with abnormal gene expression patterns have
440 been observed in the retina of *rdd* chicks at hatch (Finnegan et al., 2010). Taken together the
441 results emphasise the importance of vision to chicks and growing birds for locating and
442 ingesting food (Goodale, 1983; Bermejo and Zeigler, 1998).

443 Environmental pecking, as well as feeding and drinking, is also primarily visually
444 mediated (Green, 1998; Prescott et al., 2003). As previously demonstrated (Collins et al.,
445 2011), *beg* chicks showed less environmental pecking at week 5, and both *beg* and *rdd*
446 showed less at week 9 (Fig. 1) and in adult females (Fig. 2), compared with WL.
447 Environmental pecking was the most common behaviour performed by WL adults,
448 emphasising the importance of visual stimuli for pecking and feeding behaviour (Rogers,
449 1995; Appleby et al., 2004). However, differences in the pattern of responses for *rdd*, which
450 were similar to WL at 1 and 5 weeks, suggest that a high degree of visual ability is not as
451 critical for exploratory behaviour as for ingestion. It is, however, possible that the differences
452 in environmental pecking between the two lines of blind chickens may be linked to the
453 behaviour that was learnt while *rdd* birds had some vision during early life.

454 A higher frequency of sitting in *beg* and *rdd* at 1 and 5 weeks and in *beg* at 9 weeks
455 compared with WL may be a consequence of a relative lack of visual stimulation. The
456 significantly higher frequency of sitting behaviour in the *beg* adults than both *rdd* and sighted
457 birds may be due to the continuation of behaviour learnt as chicks in response to a lower level
458 of environmental stimulation. It is possible that the relatively high rate of walking and
459 drinking in *rdd* at 9 weeks is a sign of frustration as the visual ability of these birds was
460 clearly compromised at this age.

Abnormal behaviours occurred in *rdd* and *beg* and were not observed in WL. Abnormal behaviour such as repetitive circling is the most obvious characteristic of the blind birds and is presumed to be a response to the lack of awareness of the environment (e.g. conspecifics, potential predators and obstacles). Similarly star-gazing may be an attempt to better detect, locate and distinguish auditory stimuli that are present in the surrounding environment (Schnyder et al., 2014). The blind birds frequently collide with other birds, pen walls and equipment and collisions were usually followed by circle walking. The absence of abnormal behaviour in *rdd* to 9 weeks of age suggests that their putative limited vision was sufficient to allow them to fulfil normal orienting behaviours whereas when the birds became blind, as adults, they started to perform abnormal behaviours using their aural sense as a compensation for the inability to fulfil essential visually based orienting behaviour (Fig. 1 and 2).

The observation that *rdd* retained some vision at 9 weeks (Table 4) was unexpected and is consistent with behavioural differences and the results for Behavioural Synchrony (Bs) and Nearest Neighbour Distance (NND; Table 2). Bs declined in *rdd* to a similar level as *beg* at 9 weeks whereas NND was similar to WL until 9 weeks ($P<0.05$). It is likely that *rdd* have sufficient sight to be aware of conspecifics but are increasingly unable to differentiate different behaviours at 9 weeks. This suggests that they have extremely poor eyesight by 9 weeks of age because chickens are normally able to detect social signals even at low light intensities (Kristensen et al., 2009). In the adult birds, Bs was similar in *rdd* and *beg* and was significantly lower than in WL whereas NND did not differ among the 3 lines. The latter observation may be a consequence of the relatively small size of the pen compared with the number and size of the birds.

The welfare and ethical consequences of keeping blind chickens for commercial egg laying have been discussed elsewhere (Collins et al., 2011; Sandøe et al., 2014). Additional data from this experiment confirm the lower body weights of *beg* and *rdd* compared with WL

and contrast with those of Ali and Cheng (1985) who reported no difference between adult blind *rd* hens and sighted White Leghorn controls. Higher mortality and lower body weight in blind birds is evidence of lower welfare compared with sighted birds whether blind from hatch or becoming blind during rearing. A higher HL ratio is indicative of a greater level of stress (Gross and Siegel, 1983) and whereas the HL ratio was significantly different between the lines, the means were all relatively low and well within the normal physiological range. These data are also not consistent with Ali and Cheng (1985) who concluded that the blind-from-hatch birds were less stressed than the sighted ones partly on the basis of a lower HL ratio. Furthermore the reduced rates of behavioural synchrony and group aggregation seen in both the *rd* and *beg* adults are indicative of reduced welfare of blind hens independently of whether they are blind from hatch or become blind during early growth. The inability to perform behaviours in synchrony may reduce a positive state of welfare linked to social activities and indirectly affect the willingness to engage in other normal behaviour such as feeding.

5. Conclusions

It is concluded that abnormal behaviours are a response to a complete loss of vision, regardless of initial sight. Thus in general the behaviour of *beg* and *rd* as adults is similar. Greater drinking, walking and preening in *rd* compared with WL at 9 weeks or as adults may be a consequence of frustration at the loss of visual acuity. Behavioural synchrony was comparable in birds that are blind from hatch or have limited vision and develop blindness at a later age whereas group aggregation may be different only in birds that have totally lost their vision. Taken together, the difficulty that blind birds have in feeding and drinking and their effects on body weight, mortality and behaviour (fewer environmental pecks, lower BS and greater NND), decreased social reinstatement behaviour and lower activity in *beg*

chickens, and the increase of such effects in *rdd* as they become blind, is consistent with the view that the wellbeing of blind birds is severely compromised. The conclusion that blind birds are less stressed than sighted counterparts (Ali and Cheng, 1985) is therefore refuted.

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 583

584 Figure legends

585

586 **Fig. 1.** Back transformed means for the proportion of observations of different behaviours
587 for *beg* (black bar), *rdd* (light grey bar) and WL (dark grey bar) chickens at 1, 5 and 9 weeks
588 of age. Note the different scales within rows one and two and the large difference in the scale
589 for sitting compared with the other behaviours in the third row. Tests of significance are
590 provided for *beg* vs. *rdd* above the first column and *rdd* vs. WL over the third column for
591 each age: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

592

593 **Fig. 2.** Back transformed means for the proportion of observations of different behaviours
594 for female (left) and male (right) from three lines of adult chickens: *beg* (blind at hatch,
595 black bar), *rdd* (blind as adults, light grey bar) and WL (sighted, dark grey bar). Note the
596 different scales and the large differences in the scales for standing and pecking the
597 environment. Tests of significance are provided for *beg* vs. *rdd* above the first column and
598 *rdd* vs. WL over the third column for each gender: ** $P < 0.01$, *** $P < 0.001$, § test of *rdd* vs.
599 zero $P < 0.001$.

600

601 **Fig. 3.** Back transformed means for Nearest Neighbour Difference for female (left) and male
602 (right) chickens from three genetic lines that were blind (*beg* and *rdd*) or sighted (WL) as
603 adults. Interaction of line x gender, $F(2,5)=8.25$, $P < 0.05$.

604

605 **Fig. 4.** Back transformed means of different behaviours at 1, 5 and 9 weeks of age in three
606 lines of chicks subjected to physical isolation (black bars) or visual isolation (grey bars)
607 showing significant ($P < 0.001$) line x isolation interactions. A, latency to move from the start
608 box; B, number of lines crossed (a measure of activity); C, number of pecks and B, number of
609 peeps (potential measures of frustration); and E, percentage of scans near pen mates (a proxy

610 measure of sight). Tests of significance are provided for *beg* vs. *rdd* above the first pair and
611 *rdd* vs. WL over the second pair of columns. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

612

613 **Fig. 5.** Back transformed means for of different behaviours at 1(black bar), 5 (light grey bar)
614 and 9 (dark grey bar) weeks of age in three lines of chicks subjected to physical or visual
615 isolation showing significant ($P<0.05$) line x week interactions. A, number of pecks; B,
616 percentage of scans near pen mates. Tests of significance are provided for *beg* vs. *rdd* above
617 the first set and *rdd* vs. WL over the second set of columns. *** $P<0.001$.

618

Supplementary Table 1.

Mean and SED on the transformed scale (logit) of the proportion of different behaviors of *beg*, *rdd* and White Leghorn (WL) chicks at 1, 5 and 9 weeks of age. The WL is a normal sighted line, *beg* and *rdd* have a similar genetic background as WL but inherits a recessive gene causing blindness throughout life (*beg*), or are sighted at hatch and progressively loses sight with age (*rdd*).

Week	Line	Week			Average SED	Line	Week	Line x week
		0	5	9				
Drinking	<i>beg</i>	-4.875	-3.394	-3.625	0.4022	6.87***	3.20	5.95***
	<i>rdd</i>	-2.723	-3.238	-2.839				
	WL	-2.428	-3.390	-4.203				
Abnormal	<i>beg</i>	-5.560	-2.830	-2.920	0.7668	31.93***	17.97***	0.13
	<i>rdd</i>	0.000	-6.170	-5.850				
	WL	0.000	0.000	-6.900				
Other	<i>beg</i>	-5.354	-4.951	-4.012	0.7550	3.07	6.03**	1.96
	<i>rdd</i>	0.000	-4.178	-4.881				
	WL	-6.949	-3.599	-3.679				
Preen	<i>beg</i>	-4.648	-2.915	-2.345	0.4214	2.66	38.00***	1.12
	<i>rdd</i>	-4.289	-2.501	-1.891				
	WL	-4.747	-2.180	-2.118				
Stand	<i>beg</i>	-2.086	-2.195	-2.440	0.3231	2.14	0.27	0.25
	<i>rdd</i>	-2.274	-2.076	-2.189				
	WL	-1.857	-1.746	-1.799				
Walk/run	<i>beg</i>	-1.700	-2.317	-2.596	0.2549	8.59	7.46	6.75***
	<i>rdd</i>	-2.053	-2.144	-1.994				
	WL	-1.858	-2.068	-3.425				
Feeding	<i>beg</i>	-3.250	-1.696	-2.217	0.3143	0.91	10.69***	5.86***
	<i>rdd</i>	-1.925	-1.653	-2.032				
	WL	-1.069	-1.332	-2.526				
Peck environment	<i>beg</i>	-2.914	-2.891	-2.408	0.3666	16.92**	5.04**	0.61
	<i>rdd</i>	-2.107	-1.625	-1.703				
	WL	-1.843	-1.483	-1.002				
Siting	<i>beg</i>	0.482	-0.232	-0.110	0.2513	25.77***	7.95***	3.75**
	<i>rdd</i>	-0.079	-0.773	-0.845				
	WL	-1.158	-1.601	-0.737				

Degrees of freedom were 2, 2 and 4 for line, week and line x week interaction with denominator degrees of freedom of 6-10 for line and 87-97 for week and line x week. Tests of significance for line and week are from an analysis with no interactions. Significance levels for time were similar to those in the analysis with time and line x week. **, P<0.01; ***, P<0.001.

Supplementary Table 2.

Mean and SED on the transformed scale (logit) of the proportion of different behaviors of *beg*, *rdd* and White Leghorn (WL) male and female adult chickens. The WL is a normal sighted line, *beg* and *rdd* have a similar genetic background but the *beg* line inherits a recessive gene causing blindness throughout life, whereas *rdd* is sighted at hatch and is blind as an adult. * P<0.05; **P<0.01; ***P<0.001.

Behaviour	Breed	Gender		Avg SED	Line	Week	Line x Week
		Female	Male				
Drinking	<i>beg</i>	-2.822	-3.417	0.6029	3.79	3.67	0.57
	<i>rdd</i>	-2.048	-3.334				
	WL	-3.627	-4.096				
Abnormal	<i>beg</i>	-1.870	-1.749	0.4009	1.08	0.34	0.48
	<i>rdd</i>	-2.003	-2.463				
	WL	0.000	0.000				
Other	<i>beg</i>	-5.555	-4.475	0.9158	1.69	0.05	2.13
	<i>rdd</i>	-5.080	-4.501				
	WL	-3.264	-4.539				
Preening	<i>beg</i>	-1.642	-2.396	0.2958	4.44	6.92*	1.60
	<i>rdd</i>	-1.308	-1.401				
	WL	-1.421	-2.127				
Standing	<i>beg</i>	-1.864	-0.909	0.2641	1.77	44.29***	11.61***
	<i>rdd</i>	-2.992	-1.031				
	WL	-2.901	-0.197				
Feeding	<i>beg</i>	-2.224	-2.911	0.3032	3.04	4.85	1.47
	<i>rdd</i>	-2.125	-2.784				
	WL	-2.003	-2.026				
Walk/run	<i>beg</i>	-2.597	-1.799	0.4785	0.09	0.40	7.06*
	<i>rdd</i>	-2.209	-2.171				
	WL	-1.605	-3.287				
Peck environment	<i>beg</i>	-1.868	-2.954	0.3577	9.29**	30.30***	0.49
	<i>rdd</i>	-1.424	-2.258				
	WL	-0.661	-1.962				
Siting	<i>beg</i>	-1.539	-1.682	0.3893	4.63*	2.73	2.97
	<i>rdd</i>	-2.508	-1.999				
	WL	-3.211	-2.059				

Degrees of freedom were 2, 1 and 2 for line, gender and line x gender interaction with denominator degrees of freedom of 5-9 for line, gender and line x gender. Tests of significance for time, line and week are from an analysis with no interactions. * P<0.05; **, P<0.01; *** P<0.001.

Supplementary Table 3

Transformed (back transformed) mean latency to move, numbers of lines crossed, proportion of scans near pen mates, number of peeps and environmental pecks for *beg*, *rdd* and WL in physical or visual isolation from their pen mates at 1, 5, and 9 weeks of age.

Trait and line	Week 1		Week 5		Week 9		SED ^a	F-ratios and significance ^b					
	Physical	Visual	Physical	Visual	Physical	Visual		Line (L)	Isolation (I)	Week (W)	L x I	L x W	L x I x W
Latency to move (log s)													
<i>beg</i>	2.50	3.26	3.22	3.33	2.88	2.84	0.848	17.99 **	44.34 ***	9.07 *	12.45 ***	2.90 ns	1.31 ns
<i>rdd</i>	1.11	2.39	-0.21	0.32	0.91	2.51							
WL	-2.51	1.17	-1.32	1.54	0.09	1.86							
Lines crossed (√n)													
<i>beg</i>	3.00	2.69	2.77	2.56	2.38	3.06	0.499	34.44 **	21.56 ***	1.21 ns	41.24 ***	3.48 ns	1.70 ns
<i>rdd</i>	2.57	3.57	3.32	3.92	3.43	3.29							
WL	6.40	3.48	6.81	3.02	4.63	3.21							
Proportion near pen mate scans (logit %)													
<i>beg</i>	0.64	0.22	1.49	0.66	0.64	0.44	0.420	175.59 ***	192.79 ***	9.29 *	25.86 ***	6.72 *	0.18 ns
<i>rdd</i>	3.94	-0.01	2.86	0.38	1.64	0.41							
WL	4.61	1.85	3.32	0.48	1.75	0.86							
Number of peeps(√n)													
<i>beg</i>	7.75	8.96	2.48	5.35	3.20	4.21	0.927	35.15 **	192.84 ***	57.77	17.89 ***	1.66 ns	0.97 ns
<i>rdd</i>	6.75	13.25	3.17	9.94	2.90	6.79				***			
WL	7.13	12.45	2.02	6.95)	3.14	5.08							
Number of pecks(√n)													
<i>beg</i>	3.41	2.11	2.97	2.01	2.08	2.38	0.401	0.99 ns	375.65 ***	1.73 ns	48.20 ***	5.45 *	0.08 ns
<i>rdd</i>	4.23	1.88	3.26	1.42	2.78	1.64							
WL	4.17	0.58	4.19	1.83	4.32	1.77							

^a Maximum standard error of a difference between two means.

^b Variance ratios and significance levels are indicated for the main effects and interaction; degrees of freedom were 2,4 for L and A; 1,12 for I; 2,12 for L x I and 4,8 for L x A and L x A x I.

* P<0.05; ** P<0.01; *** P<0.001; ns = not significant.

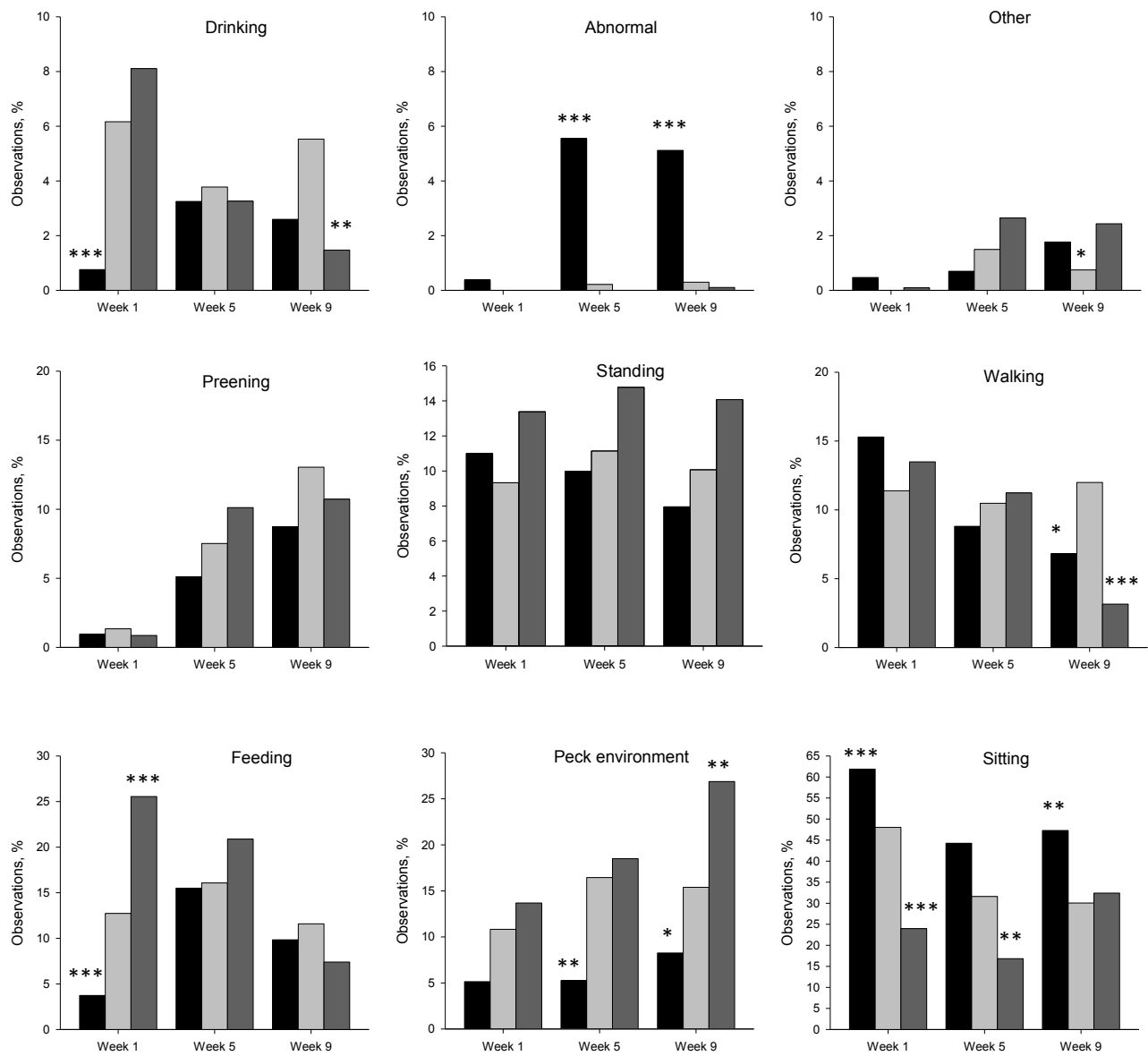


Figure 1.

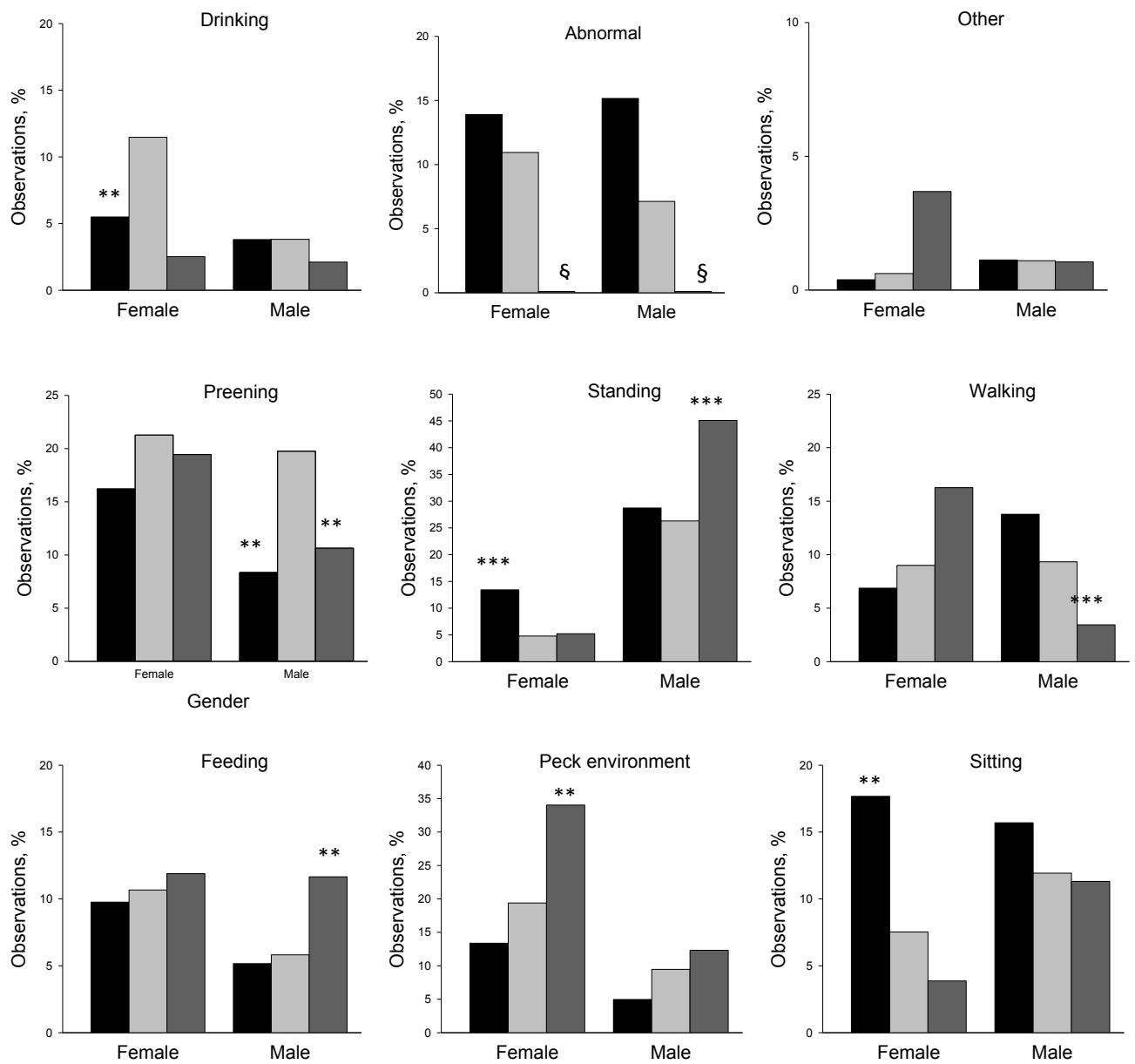


Figure 2.

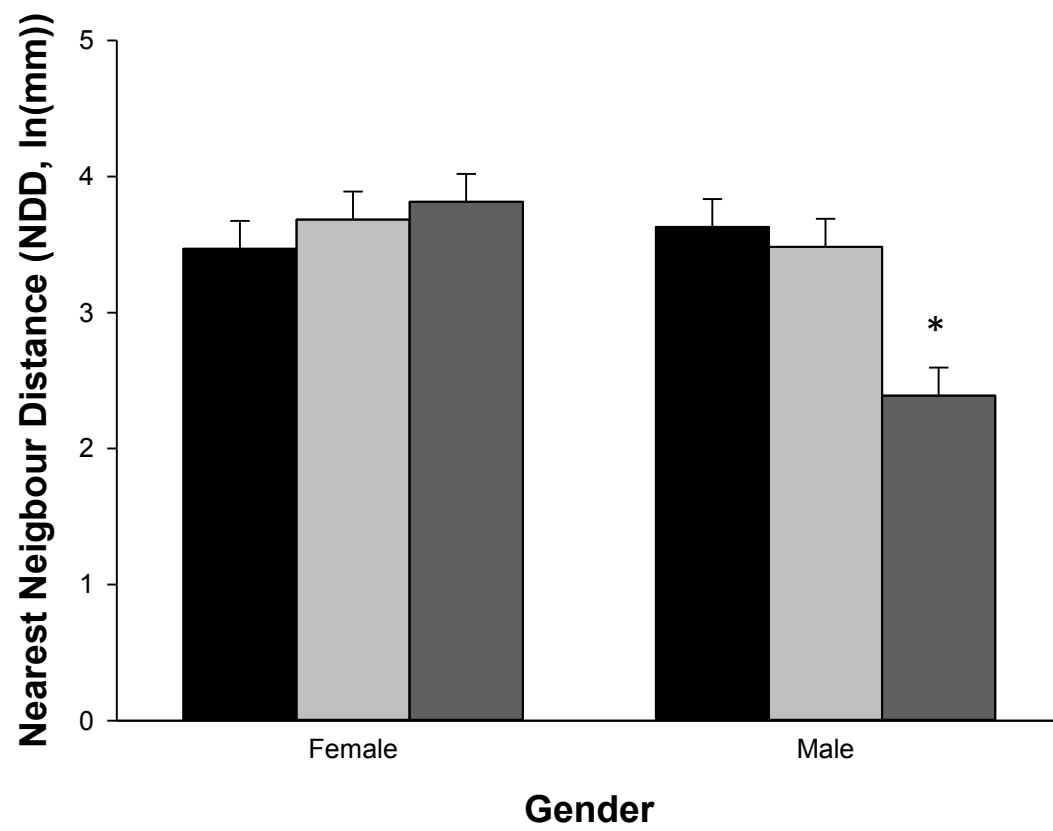


Figure 3.

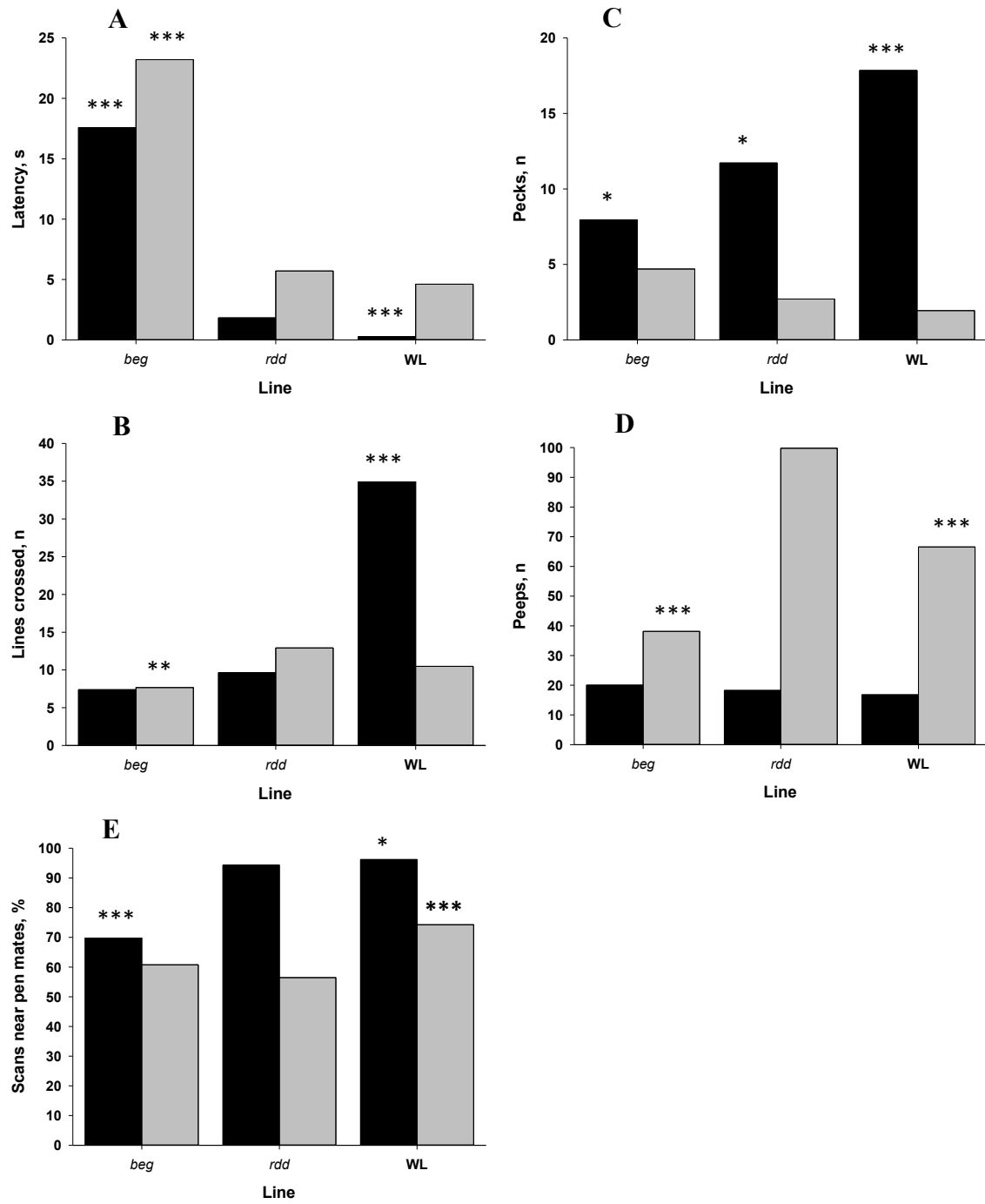


Figure 4.

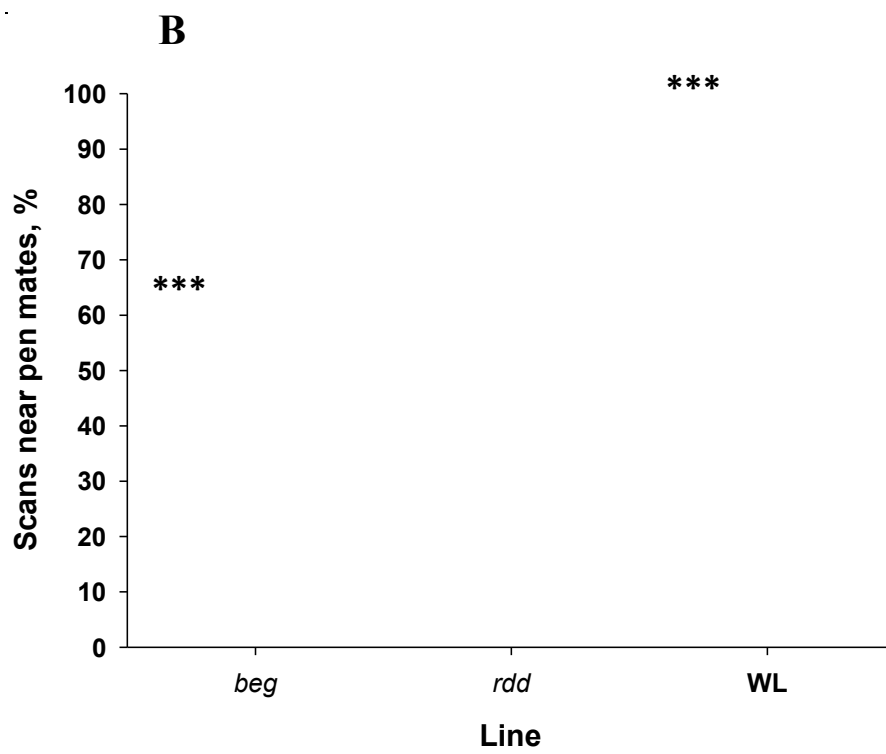
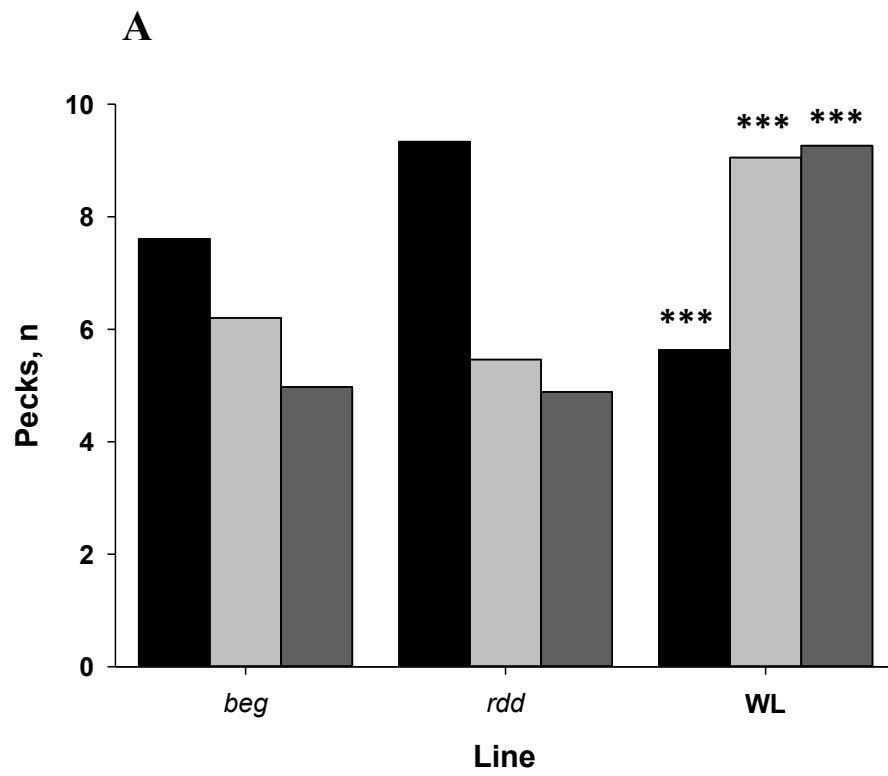


Figure 5.